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SYNTHESIS AND CHARACTERIZATION OF NEW ISATIN DERIVATIVES FOR CYTOTOTOXIC ACTIVITY

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ABSTRACT

A series of wereN-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamides were synthesized by treating (5-benzoyl-1H-benzo[d]imidazol-2-yl) carbamatewith different substituted isatins. The novel compounds were characterized on the basis of spectral (FT-IR, ^{1}H NMR, Mass) analysis. All the synthesized derivatives were screened for cytotoxic activity against Hela cancer cell lines using MTT assay. All the synthesized compounds produced a dose dependent inhibition of growth of the cells. The IC_{50} values of all the synthetic test compounds were found between 17.32-55.57. The potency of (IC_{50} values) of cytotoxic activit of compounds was compared with that of known cytotoxic agent, Cisplatin. Almost all the synthesized novel compounds showed the most potent activity against all cell lines. These results indicate that C-5 substituted derivatives may be useful leads for anticancer drug development in future.

KEY WORDS

Isatin, 1, 3, 4-oxadiazole, cytotoxic activity, MTT assay.

INTRODUCTION

It is very evident that, according to several patents and reports, a continuous attempt is being made to synthesize the various heterocyclic moieties of high efficiency and minimum toxicity. Among them the indole nucleus has been reported to possess great importance in the field of medicine and biochemistry.[1-5]

A dose of anticancer drug sufficient to kill tumor cells often toxic to the normal tissues and leads to many side effects, which in turn limits its treatment efficacy[6].In this study we have synthesized thirteen compounds and were evaluated for their anticancer activity against Hela (Human cervical carcinoma), $HEPG_2$ (Hepato cellular carcinoma) and HCT-116(Human colon carcinoma) cancer cell lines.

All the chemicals and solvents (M/s Sigma Aldrich/S.D. Fine chemical/Loba) were purchased from local vendors and solvents were purified before being used.Pre coatedsilica gel F_{254} (Merck) were employed to check the TLC for the reaction progress and purity. Melting points were recorded in open glass capillaries using *Thomas Hoover* melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR spectrophotometer in KBr pellet.Massspectra were obtained on VG-7070H mass spectrometer and ^{1}HNMR spectra were recorded at 300MHz on Bruker Advance NM spectrometer in CDCl3(7.26)or DMSO-d6(2.49).Chemical shifts are expressed in (ppm) relative to TMS an internal standard.

EXPERIMENTAL METHOD:

MATERIALS &METHODS

I. Synthesis of Indole-2, 3-diones (Isatins,)

a) Isonitrosoacetanilide – General Procedure:

In a 5 lit. R.B. flask were placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300gm) followed by a solution of an appropriate aromatic amine in 300ml of water and concentrated hydrochloric acid (0.52mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

b) Indole-2,3-diones – General Procedure :

Sulphuric acid (600g, d:1.84, 326 ml) was warmed at 50°C in a one litre RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (0.46 mol) was added at such a rate so as to maintain the temperature between 60°C to 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80°C and maintained at that temperature for 10 minutes, to complete the reaction. Then the reaction mixture was cooled to room temperature and poured onto crushed ice (2.5 kg) while stirring.

II. Synthesis of N-(6-benzoyl-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide

A mixture of methyl (5-benzoyl-1H-benzo[d]imidazol-2-yl) carbamate add 0.01mole of hydrazine hydrate (99%) were taken in 20ml of methanol, heated under reflux on a water bath for 2 hrs.The alcohol was reduced to half of its volume and cooled. The product separated was filtered and small portions of cold alcohol first and then with cold water repeatedly and dried. The product purified by recrystallization from methanol has resulted white solid.

III. Synthesis of N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamide (III)

The N-(6-benzoyl-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (0.01mol) and appropriate isatin (0.01mol) in methanol (20ml) 2 drops of glacial acetic acid heated under reflux on water bath for 8-12 hrs. The product thus obtained was filtered, washed with water

CHEMICALS:

Fetal bovine serum (FBS),Dulbecco's modified eagle's medium(DMEM),pencillin,amphotericin B and streptomycin were purchased from Himedia(Mumbai,India) MTT (3-(4,5-dimethylthiazol-2yl)-2,5diphenyltetrazolium bromide) was purchased from Sigma Aldrich Compony, USa.Cisplatin was bprocured from local market with trade name as cytoplatin 50mg/50ml marketed by CiplaPvt Ltd, Ahmedabad ,India, Himedia,Mumbai,India.

CELL CULTURE:

The cell cultures like HELAwere procured from National Center for Cell Sciences [NCCS], Pune, India.These cells lines were grown in culture and maintained using suitable media (DMEM) and were grown in culture medium supplemented with 10% fetal bovine serum,1%L-glutamate and 1%penciline-streptomycin-amphotericin-B-

antibiotic solution. Cells were seeded in 25cm^2 tissues culture flasks[Tarsons,Mumbai,INDIA] at250,000 cells\flask in a total volume of 9Ml.when confluent ,all the cells were trypsinized and seeded in 96-well tissue culture plates [Tarsons,Mumbai,INDIA]

IN VITRO CYTOTOXIC ACTIVITY:

In-vitro anticancer activity against MCF-7(Breast), Hela (Human cervical carcinoma), $HEPG_2$ (Hepato cellular carcinoma) and HCT-116(Human colon carcinoma) cancer cell lines were determined using 96 well tissue culture plates. The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium. The drug solution were serially diluted at concentration of 10µg/ml to100µg/ml with complete growth medium containing 1µg/ml, 3µg/ml, 10µg/ml,30µg/ml and 100µg/ml concentrations(<2%DMSO solution).The 100µl of cell suspension was added to each well of 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37^{o}C, 5% CO_2, 90 %relative humidity) for 24 hrs. The test drug solutions in complete growth medium (100µl) were added after 24hrs incubation to the wells containing cell suspension. After 48hrs of treatment with different concentrations of test drug solution, the cells were incubated with 20µl of MTT (2.5mg/mL) for 2 hrs. After 24 hrs medium was removed and 80µl of lysis buffer was added to each well the plate was wrapped in aluminum foil to prevent the oxidation of the dye and the plate was placed on a shaker for overnight.7. The absorbencies were recorded on the ELISA reader at 562nm wavelength. The absorbance of the test was compared with that of DMSO control to get the % inhibition. The cytotoxic effects of the compounds were calculated as percentage inhibition in cell growth as per the formula.[2] % cytotoxicity=1-[(O.D.in sample well)]/O.D. in control well)] ×100.

PHYSICAL DATA OF N-(6-BENZOYL-1H-BENZO [D] IMIDAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDENE) HYDRAZINE CARBOXAMIDE

Compound	Substituents (R)	m.r (^{0}C)	Yield (%)	Mol.Wt
1a	H	265	70	423
1b	5-CH_3	270	75	437
1c	7-CH_3	272	72	437
Id	5-F	260	85	439
1e	5-$COOCH_3$	280	69	431
1f	5-CL	285	70	457
1g	7-CL	279	82	429
1h	5-Br	284	84	402
Ii	5-No_2	285	76	468
1j	7-No_2	270	81	468
1 k	5-COOH	272	79	432
1i	7-$COOCH_3$	273	80	437

IN VITRO CYTOTOXIC ACTIVITY OF N-(6-BENZOYL-1H-BENZO [D] IMIDAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDENE) HYDRAZINE CARBOXAMIDE)

S.No.	Compound	R	IC_{50}(µg/ml)
1	A	H	26.83
2	B	5-CH_3	41.48
3	C	7-CH_3	34.83
4	D	5-F	28.16
5	E	5-$COOCH_3$	17.321
6	F	5-Cl	41.48
7	G	7-Cl	25.7 9
8	H	5-Br	55.57
9	I	6-Br	52.32
10	J	5-NO_2	31.10
11	K	7-NO_2	31.081
12	L	5-COOH	32.42
13	M	7-$COOCH_3$	26.83
14	Standard	Cisplatin	14.08

RESULTS AND DISCUSSION

In present study, the N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamide (III a-m). Compounds were synthesized as depicted in the Scheme. The thirteen different novel compounds were prepared. The preparation of title derivatives is outlined in scheme. The physical data of the all synthesized compounds were purified by column chromatography. The thirteen compounds were tested for their *in vitro* cytotoxic activity against Hela (Human cervical carcinoma) cancer cell lines by using MTT assay method. The results were satisfactory. Among all the compounds IV M(R=5-COOH3) was effective against the entire cell line.

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